The multiple faces of the human immune system

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THE MULTIPLE FACES OF THE HUMAN IMMUNE SYSTEM

Modern life causes low-grade inflammation and thereby provokes conflict between the selfish immune system and the selfish brain

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Having a human immune system is like having a life insurance.

If it pays out it is too late.

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Introduction

This thesis focuses on the human immune system as the ultimate organ explaining the development of most, if not all, 'typically Western' chronic diseases. Patho-physiologically, the emphasis is on the development of 'systemic low-grade inflammation' (LGI). A red thread throughout this thesis is Charles Darwin's (1809-1882) 'adaptation to the conditions of existence'. We explain our physiology and functioning in the light of evolution, being the only approach that really 'makes sense in biology' (Theodosius Dobzhansky, 1900-1975).

During millions years of evolution the immune system has saved simple organisms, such as the lamprey, and later on much more complex types of life, including humans and plants, although the latter make use of a much less complicated innate-like immune system that e.g. does not rely on mobile defence cells. Analogous to all other systems, the immune system has been adapted by evolutionary pressure to the benefit of survival and reproduction. There is good evidence to show that infection should be considered as the 'roller coaster' of evolution that shaped all forms of life, and humans in particular. Even today it are still infections by parasites, bacteria, fungi and viruses causing the most devastating conditions in humans. Contemporary examples are Zika in the America's, ebola outbreak in Africa, HIV affecting millions of humans, salmonella infections worldwide and Aspergillus in intensive care units (Bellet 2013, Tyghe 2011, Wingard 2008).

For example, about half of the world's population is at risk of malaria infection, causing about 438,000 deaths on a yearly basis (WHO *http://www.who.int/features/factfiles/malaria/en/*). The malaria parasite might be at least 200 million years old, as compared to the about 160,000 years of homo sapiens. The battle between the two species has become intensified from about 80,000 years ago, i.e. long after homo sapiens started to leave Africa some 100,000 years ago (Cserti 2007). According to Darwin's 'adaptation to the conditions of existence' this battle has shaped both the genomes of Plasmodium and modern humans. Some examples of human adaptations by positive selection of polymorphisms are given in the Table 1 (Kwiatkowsi 2005). At least some of these adaptations, like sickle haemoglobin, confer heterozygote advantage, also named balancing selection, with homozygotes exhibiting (serious) disease, while heterozygotes are not even fully protected. The existence of haplotypes witness the independence of the occurrence and preservation of the sickle haemoglobin mutation in at least 4 malaria-endemic regions in the world, adding to the notion of the very strong selective pressure that has and is still exerted by Plasmodium.

Table 1. Anti-malarial strategies related to malaria survival.

Membrane proteins	Duffy antigen (chemokine receptor functioning as recognition site, confers resistance to P. vivax). Glycophorin. Chloride/HCO3- exchanger (ovalocytosis). Blood group resistance: 0>B>A (South America 100% type 0).
(Energy) metabolism	Glucose-6-phosphate dehydrogenase (G6PD; higher RBC oxidative stress).
Hemoglobin	Hb structural variants (examples HbS, HbC, HbE). Thalassemias (notably alpha, beta).
Haptoglobulin	Binding of free Hb.
Immunity, inflammation, adhesion	Ag presentation (HLA), cytokines (ILs, interferon, TNF-alpha), NO-synthase, adhesion molecules (ICAM1).

Humans have conquered the world and survived their continuing search of novelty (Pruimboom 2015). Encountered challenges included e.g. climate, famine, thirst, but most of all infections (Nunn 2014). The corresponding evolutionary pressure triggered many phenotypic adjustments by either genetic or epigenetic modifications. As an example we mention 3 major strategies:

1. A shift from strong to smart (Watve 2007), needed to 'outthink' much stronger animals and to guarantee food, water, shelter from cold and heat, protection from violence, and solutions for infections (Nunn 2014)

2. A shift from strong to smart leading to the highest encephalization quotient among all animals (Roth 2005) and a change from present to future thinking through the development of a more dopamine-oriented neuroanatomy (Vernier 2004)

3. A highly adequate-functioning immune system, though expensive, needed to survive pathogenic load and novel environmental challenges, prior to the institution of hygiene and modern medicine (e.g. antibiotics).

A highly effective 'somatic immune system' has provided humans with many fitness benefits that are nevertheless endowed with important limitations and costs. A reactive pro-inflammatory immune response is metabolically expensive; not only from a caloric point of view, but also from the view of nutrients, such as calcium and magnesium. Calcium, as signal transducer, is essential for adequate functioning of the immune system, muscles, brain and others. The immune system's activation in general (Vig 2009), and more specifically of T-cells, induces profound increases of intracellular calcium, needed to initiate the production of

cytotoxic substances and T-cell antigen receptor ligation. Sickness syndrome may accompany infection and the concomitant lack of mobilization causes loss of calcium from bones and augmented urinary calcium excretion, illustrating 'use it or lose it'. The concomitant anorexia of severe infection causes long-term reduced intestinal calcium and magnesium intakes, and may, aggravated by vitamin D and K deficiencies, add to the negative calcium balance that ultimately results in osteopenia (Straub, J Intern Med 2010).

Thus, the immune system's actions can be highly debilitating and damaging in the face of its causation of sickness behaviour, cytotoxicity, fever, fatigue and micronutrient depletion, all of which may ultimately lead to multiple organ failure, including damage to the immune system itself. Prevention of immune responses are therefore of paramount importance. A unique part of the human immune system is the 'behavioural immune system' (Schaller 2011), since it aims at the predictable benefits of prevention. This system developed to avoid pathogen contact, thereby preventing an energy-costly reactive response and saving energy and resources for other essential organs, including the brain and the heart (Schaller 2011, Park 2007).

The human brain and the immune system have become so important for human physiology and evolutionary fitness that both systems have developed selfish behaviours by their capabilities to pull on energy and other resources, with the solemn purpose to maintain their own anatomies and optimal functioning (see for the selfish brain concept: Kubera 2012, Peters 2011, Peters 2009, Peters 2007, Peters 2004 and for the selfish immune system: Straub 2014, Pruimboom 2015). Upon challenge, an optimal human response therefore depends on the flexibility to (re)distribute energy, and thereby in first instance prevent energy conflicts between the brain and the immune system. The circadian rhythm (Figure 1), allowing the brain to dominate daytimes and the immune system to exploit night times, illustrates how evolution shaped collaboration between these two highly selfish systems, while avoiding conflict (Straub 2011; Pacheco-Lopez 2011). The selfish behaviour of the immune system may, from a pathophysiological point of view, explain why LGI disturbs the functioning of many other organs, including the brain. LGI puts the immune system on top of hierarchic priorities as e.g. evidenced by the observation that the system even endorses breakdown of an essential muscle like the diaphragm (Hafner 2014).

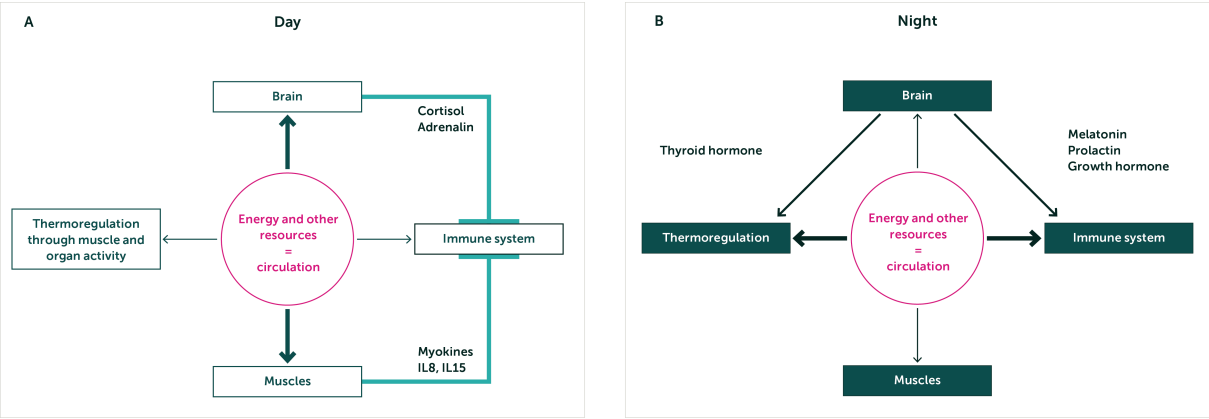

Figure 1. Circadian fuel allocation.

At daytime (A): the immunosuppressive stress hormones, cortisol and adrenalin, together with anti-inflammatory myokines, shut-off the immune system. At night (B): growth hormone, prolactin and melatonin, secreted shortly after sleep onset, activate the immune system. Modified from Pacheco-Lopez (Philos Trans R Soc Lond Sci 2011).

Known risk factors for LGI and chronic disease are age, smoking, socioeconomic status, obesity, chronic psychosocial stress, sedentary lifestyle, toxins, insufficient sleep, nutritional factors (nutrient composition, time, frequency) and abuse of legal and illegal drugs, alcohol included (Schmid 2015, Henson 2015, Ruiz 2014, Ruiz 2013, Egger 2012a, Egger 2012b, Egger 2011). These, mostly environmentally-driven, risk factors seem inevitable in current Western societies and their shares and intensities are most likely destined to further increase in the future. Importantly, many of these risk factors exhibit interaction, while contemporary humans are likely to suffer from them in concert. This current 'condition of existence', occurs as opposed to the stress factors experienced by humans living in the environment of our ancestors. In that environment, they had to cope with short-term mono-metabolic danger factors (e.g. hunger, thirst, cold, heat), whereas modern humans are exposed to multi-metabolic risk factors that stimulate an energy conflict between organs and major systems (Wells 2012). The ensuing conflict between what we currently experience and to what our genes are adapted is the basis of the so-called 'mismatch hypothesis' of 'typically Western' diseases.

Mono-metabolic stress factors have shaped mechanisms for survival and reproduction, such as short-lasting infections, insulin resistance, activation of the sympathetic nervous system and others. All of these responses emerged with the purpose to first protect the brain from damage and energy deficits (Pruimboom 2011). Mono-metabolic challenge increases basal metabolic rate and frequent challenges in a resource-restricted (thrifty) environment prevent a pathological increase of adipose tissue size. A sizeable fat-mass in concert with other risk factors, is not unlikely to become an inflammatory compartment (Exley 2015). Multi-metabolic risk factors may, on the other hand, cause LGI and a hypo-metabolic state (Wells 2012). The latter is probably the main reason for the deleterious effects of LGI, since a hypo-

metabolic state causes energy deficits in multiple organs, and consequently multi-organ damage (Straub 2010).

While among our ancestors, cold, heat, starvation and repetitive infections were major causes of death, exposure to mild cold, mild heat, short fasting periods and the regular consumption of small amounts of 'toxic' nutrients provided hormetic triggers. Mildly toxic insults, for example, derive from plant secondary metabolites, many with bitter tastes. The discovery of the nrf2 receptor has revolutionized toxicology by unveiling the benefits of low amounts of toxins (Bryan 2013, Hayes 2014; Forman 2014). Ultimately, it is all about hormesis: every dose-response curve is U-shaped (Calabrese 2014), as opposed to the saturation curves that are usually depicted in textbooks and lectures, and is the first to pop-up when entered into Google-pictures. Establishment of 'dietary reference intakes' (e.g. AIs, RDAs, ULs) have for long used dose-response curves showing a range starting from deficiency, via adequacy, to toxicity, but the step to 'what does not kill you makes you stronger' has only recently become appreciated. This notion deserves rethinking of the definition of 'essential nutrients'.

Mild triggers might at least in part recover physiologic and metabolic dysfunctioning in patients suffering from 'typically Western' diseases (Mattson 2015). In other words: they may provide low-cost opportunities for secondary prevention. Conversely, the chronic absence of mild stress factors may have rendered modern 21st century humans less resistant to major toxic insults and susceptible to the development of many, 'typically Western', chronic diseases of affluence, including metabolic disorders, some types of cancer, depression and cardiovascular diseases (Mattson 2015, Calabrese 2015, Mattson 2014). Re-introduction of exposure provides low-cost opportunities for primary prevention with huge favourable potential for the society as a whole. Many changes in lifestyle are involved and their adoption is not necessarily unpleasant, as is frequently claimed. For instance, a recent study suggested that men taking sauna bathing sessions at a frequency of 4-7 times/week have 63% lower risk of all-cause and CVD mortality, compared with those having one sauna session/week. There was also a significant trend of lower fatal CVD mortality of 19 minutes sessions, compared with sessions lasting less than 11 minutes (Laukkanen 2015). A sauna session may be regarded as a mild, heat-based, stress factor with hormetic actions and broad protecting ability from the insults of the 21st century environment (Sarup 2014, Rattan 2009).

Aim of the thesis

The purpose of this thesis was to contribute to the notion that the human immune system may be selfish and perhaps even more selfish than the human brain. The exploitable part of this knowledge is that the triggering of the immune system's selfish behaviour might be responsible for most, if not all, current chronic non-communicable diseases (CNCD), and that

a chronically activated immune system produces a long-term hypo-metabolic state.

Along these lines, the aim of our field study (Chapter 3) was to show that metabolism and normal immunological functioning of contemporary humans can be recovered by 'suffering' from ancient mild stress factors, analogous to what our ancestors have experienced in the past, and as opposed to the LGI ensuing from modern life. We definitely do not propose to return to the 'caveman's life', like popular 'counterarguments' tend to posit, but to exploit the positive effects of evolutionary-based lifestyle challenges to the benefit of our current health. The only realistic and affordable road to 'healthy aging' is comprised in the 'adaptations to the past conditions of existence' according to culture of the 21st century (Cordain 2005). For this we hypothesize that living a life with short-term mild stress factors could prevent metabolic diseases and LGI (primary prevention) and even cure patients suffering from CNCD, at least to some extent (secondary prevention). In other words: short-term mild stress factors may protect and even cure modern humans from the deleterious effects of Western lifestyle.

Chapter 1 reviews LGI as the major cause of most, if not all, CNCD. Paragraph 1.1 describes the 'selfish immune system' concept, as compiled from the existing scientific literature. Paragraphs 1.2 and 1.3 review the current lifestyle factors held responsible for the development of LGI and CNCD, respectively. In paragraph 1.4 we describe several pathways leading to LGI and ensuing from modern lifestyle factors. Paragraph 1.5 discusses the gluten in grains as a specific, uniformly present, nutritional factor that is likely to be involved in the development of LGI. Paragraph 1.6 discusses the background of genetic adaptations triggered by the consumption of non-human milk and starchy-rich foods, which are two recently introduced foods into homo sapiens' diet. Our hypothesis in this paragraph may illustrate the widely misunderstood distinction between ultimate and proximate explanations of our physiology, that nevertheless in both explanations ensue from evolutionary pressure with the aim to survive and reproduce. The question here is not 'how' we adapted (proximate explanation) but 'to what' and 'why' (evolutionary approach). Chapter 2 deals with physical activity. Paragraphs 2.1 and 2.2 discuss the impact of sedentary lifestyle and a lack of physical activity on the developments of LGI and CNCD. Finally, in Chapter 3, we present our field study aiming to show that a concerted lifestyle 'intervention', based on what made us humans, may favourably affect both our metabolism and immune functioning.

References

1. Bellet, M. M., Deriu, E., Liu, J. Z., Grimaldi, B., et al. Circadian clock regulates the host response to Salmonella. Proceedings of the National Academy of Sciences 110, 9897-9902 (2013).
2. Bryan, H. K., Olayanju, A., Goldring, C. E. & Park, B. K. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. Biochemical Pharmacology 85, 705-717 (2013).
3. Calabrese, E. J. Model Uncertainty via the Integration of Hormesis and LNT as the Default in Cancer Risk Assessment. Dose-Response 13, (2015).
4. Cserti, C. M. & Dzik, W. H. The ABO blood group system and Plasmodium falciparum malaria. Blood 110, 2250-2258 (2007).
5. Egger, G. Obesity, Chronic Disease, and Economic Growth: A Case for "Big Picture" Prevention. Advances in Preventive Medicine 2011, 1-6 (2011).
6. Egger, G., Swinburn, B. & Amirul Islam, M. Economic growth and obesity: An interesting relationship with world-wide implications. Economics & Human Biology 10, 147-153 (2012).
7. Egger, G. In Search of a Germ Theory Equivalent for Chronic Disease. Preventing Chronic Disease (2012).
8. Exley, M. A., Hand, L., O'Shea, D. & Lynch, L. Interplay between the immune system and adipose tissue in obesity. Journal of Endocrinology 223, R41-R48 (2014).
9. Feske, S. Calcium signalling in lymphocyte activation and disease. Nat Rev Immunol 7, 690-702 (2007).
10. Hafner, S., Radermacher, P., Frick, M., Dietl, P. & Calzia, E. Hyperglycemia, oxidative stress, and the diaphragm: a link between chronic co-morbidity and acute stress? Critical Care 18, 149 (2014).
11. Hanson, L. A., Zahn, E. A., Wild, S. R., Döpfer, D., et al. Estimating global mortality from potentially foodborne diseases: an analysis using vital registration data. Population Health Metrics 10, 5 (2012).
12. Hayes, J. D. & Dinkova-Kostova, A. T. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends in Biochemical Sciences 39, 199-218 (2014).
13. HENSON, J., EDWARDSON, C. L., MORGAN, B., HORSFIELD, M. A., et al. Associations of Sedentary Time with Fat Distribution in a High-Risk Population. Medicine & Science in Sports & Exercise 47, 1727-1734 (2015).
14. Joseph, N., Reicher, B. & Barda-Saad, M. The calcium feedback loop and T cell activation: How cytoskeleton networks control intracellular calcium flux. Biochimica et Biophysica Acta (BBA) - Biomembranes 1838, 557-568 (2014).

15. Kubera, B., Hubold, C., Zug, S., Wischnath, H., et al. The brain's supply and demand in obesity. Frontiers in Neuroenergetics 4, (2012).
16. Kwiatkowski, D. P. How malaria has affected the human genome and what human genetics can teach us about malaria. The American Journal of Human Genetics 77, 171-192 (2005).
17. Laukkanen, T., Khan, H., Zaccardi, F. & Laukkanen, J. A. Association Between Sauna Bathing and Fatal Cardiovascular and All-Cause Mortality Events. JAMA Internal Medicine 175, 542 (2015).
18. Mattson, M. P. Challenging Oneself Intermittently to Improve Health. Dose-Response 12, 600-618 (2014).
19. Mattson, M. P. Lifelong brain health is a lifelong challenge: from evolutionary principles to empirical evidence. Ageing research reviews 20, 37-45 (2015).
20. Nunn, A. V. W., Guy, G. W. & Bell, J. D. The intelligence paradox; will ET get the metabolic syndrome? Lessons from and for Earth. Nutrition & Metabolism 11, 34 (2014).
21. Pacheco-Lopez, G. & Bermudez-Rattoni, F. Brain-immune interactions and the neural basis of disease-avoidant ingestive behaviour. Philosophical Transactions of the Royal Society B: Biological Sciences 366, 3389-3405 (2011).
22. Park, J. H., Schaller, M. & Crandall, C. S. Pathogen-avoidance mechanisms and the stigmatization of obese people. Evolution and Human Behavior 28, 410-414 (2007).
23. Peters, A., Schweiger, U., Pellerin, L., Hubold, C., et al. The selfish brain: competition for energy resources. Neuroscience & Biobehavioral Reviews 28, 143-180 (2004).
24. Peters, A., Pellerin, L., Dallman, M. F., Oltmanns, K. M., et al. Causes of obesity: looking beyond the hypothalamus. Progress in neurobiology 81, 61-88 (2007).
25. Peters, A. Build-ups in the supply chain of the brain: on the neuroenergetic cause of obesity and type 2 diabetes. Frontiers in Neuroenergetics 1, (2009).
26. Peters, A. The selfish brain: Competition for energy resources. Am. J. Hum. Biol. 23, 29-34 (2010).
27. Peters, A. & McEwen, B. S. Stress habituation, body shape and cardiovascular mortality. Neuroscience & Biobehavioral Reviews 56, 139-150 (2015).
28. Pruimboom, L. Physical inactivity is a disease synonymous for a non-permissive brain disorder. Medical Hypotheses 77, 708-713 (2011).
29. Rattan, S. I. S., Fernandes, R. A., Demirovic, D., Dymek, B. & Lima, C. F. Heat Stress and Hormetin-Induced Hormesis in Human Cells: Effects on Aging, Wound Healing, Angiogenesis, and Differentiation. Dose-Response 7, 90-103 (2008).
30. Roth, G. & Dicke, U. Evolution of the brain and intelligence. Trends in cognitive sciences 9, 250-257 (2005).
31. Ruiz-Núñez, B., Pruimboom, L., Dijck-Brouwer, D. J. & Muskiet, F. A. Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of

chronic systemic low-grade inflammation in an evolutionary context. The Journal of Nutritional Biochemistry 24, 1183-1201 (2013).

32. Ruiz-Núñez, B., Kuipers, R. S., Luxwolda, M. F., De Graaf, D. J., et al. Saturated fatty acid (SFA) status and SFA intake exhibit different relations with serum total cholesterol and lipoprotein cholesterol: a mechanistic explanation centered around lifestyle-induced low-grade inflammation. The Journal of Nutritional Biochemistry 25, 304-312 (2014).
33. Sarup, P., Sørensen, P. & Loeschcke, V. The long-term effects of a life-prolonging heat treatment on the Drosophila melanogaster transcriptome suggest that heat shock proteins extend lifespan. Experimental Gerontology 50, 34-39 (2014).
34. Schaller, M. The behavioural immune system and the psychology of human sociality. Philosophical Transactions of the Royal Society of London B: Biological Sciences 366, 3418-3426 (2011).
35. Schmid, D., Ricci, C. & Leitzmann, M. F. Associations of Objectively Assessed Physical Activity and Sedentary Time with All-Cause Mortality in US Adults: The NHANES Study. PLOS ONE 10, e0119591 (2015).
36. Schwarz, E. C., Qu, B. & Hoth, M. Calcium, cancer and killing: The role of calcium in killing cancer cells by cytotoxic T lymphocytes and natural killer cells. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1833, 1603-1611 (2013).
37. Straub, R. H., Cutolo, M., Buttgereit, F. & Pongratz, G. Energy regulation and neuroendocrine-immune control in chronic inflammatory diseases. J Intern Med 267, 543-560 (2010).
38. Straub, R. H. Neuroendokrinimmunologie: Neue Aspekte zur Pathogenese und klinischen Anwendung (Leitthema). Zeitschrift für Rheumatologie 70, 767-774 (2011).
39. Straub, R. H. Systemic disease sequelae in chronic inflammatory diseases and chronic psychological stress: comparison and pathophysiological model. Ann. N.Y. Acad. Sci. 1318, 7-17 (2014).
40. VERNIER, P. The Degeneration of Dopamine Neurons in Parkinson's Disease: Insights from Embryology and Evolution of the Mesostriatocortical System. Annals of the New York Academy of Sciences 1035, 231-249 (2004).
41. Vig, M. & Kinet, J. -P. Calcium signaling in immune cells. Nature immunology 10, 21-27 (2009).
42. Watve, M. G. & Yajnik, C. S. Evolutionary origins of insulin resistance: a behavioral switch hypothesis. BMC Evolutionary Biology 7, 61 (2007).
43. Wells, J. C. K. The evolution of human adiposity and obesity: where did it all go wrong? Disease Models & Mechanisms 5, 595-607 (2012).
44. Wingard, J. R., Ribaud, P., Schlamm, H. T. & Herbrecht, R. Changes in causes of death over time after treatment for invasive aspergillosis. Cancer 112, 2309-2312 (2008).